Disease marker combination enhances patient characterization in the Finnish sarcoidosis patients

Lahtela, E.

2017-11

Lahtela , E , Wolin , A , Pietinalho , A , Lokki , M -L & Selroos , O 2017 , ' Disease marker combination enhances patient characterization in the Finnish sarcoidosis patients ' , Respiratory Medicine , vol. 132 , pp. 92-94 . https://doi.org/10.1016/j.rmed.2017.09.014

http://hdl.handle.net/10138/298186
https://doi.org/10.1016/j.rmed.2017.09.014

publishedVersion

Respiratory Medicine 132 (2017) 92–94

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Short communication

Disease marker combination enhances patient characterization in the Finnish sarcoidosis patients

E. Lahtela [a, *], A. Wolin [a], A. Pietinalho [b], M.-L. Lokki [a], O. Selroos [c]

[a] Transplantation Laboratory, Medicum, University of Helsinki, Finland
[b] Raasepori Health Care Centre, Raasepori, Finland
[c] University of Helsinki, Helsinki, Finland

ARTICLE INFO

Article history:
Received 15 February 2017
Received in revised form
28 September 2017
Accepted 30 September 2017
Available online 3 October 2017

Keywords:
Angiotensin converting enzyme (ACE)
Human leukocyte antigen (HLA)
Prognosis
Sarcoidosis

ABSTRACT

Sarcoidosis is an inflammatory disease of unknown etiology and multiple clinical phenotypes. Clinical manifestations range from asymptomatic disease to severe loss-of-function leading to the hypothesis that sarcoidosis might not be just one disease, but consists of several distinct disease entities each with potentially distinct genetic associations. We have previously demonstrated that in our series HLADRB1* 03:01 and haplotype HLA-DRB1*04:01-DPB1*04:01 are associated with good prognosis sarcoidosis. In our recent work, we found a novel SNP (rs9905945) in the 5′upstream region of the ACE gene to be associated with favorable disease prognosis as well. The main objective of this study was to expand the previous results and analyse combined influence of the found ACE SNP rs9905945 with the protective HLA markers HLADRB1* 03:01 and HLA-DRB1*04:01-DPB1*04:01 in 188 Finnish sarcoidosis patients (resolved disease, n = 90; persistent disease, n = 98). When combining the frequencies of the rs9905945 and of the HLA markers, the strongest association was found for a combination of either/or both HLA markers and rs9905945 for good disease prognosis (37.1% in resolved vs. 11.3% in persistent, $p < 0.001$, OR = 4.61, (95%CI 2.15–9.86)). In conclusion, we discovered that a combination of the ACE SNP rs9905945 and HLA markers enhance the accuracy for predicting disease course in Finnish sarcoidosis patients further characterizing genetic differences between Finnish sarcoidosis patients with different prognosis.

To the Editor

Sarcoidosis is an inflammatory disease of unknown etiology and multiple clinical phenotypes. Clinical manifestations range from asymptomatic disease to severe loss-of-function leading to the hypothesis that sarcoidosis might not be just one disease, but consists of several distinct disease entities each with potentially distinct genetic associations [1]. This explains why no single gene or immunological pathway defect has been found. Instead, a wide range of genes have been identified, each contributing to relatively minor effects and to a variety of clinical manifestations and differences in prognosis. Nevertheless, there is strong evidence of genetic influence in sarcoidosis, proposed by family studies and the varying disease incidences in different ethnic groups [2,3].

One of the strongest genetic associations with sarcoidosis has been found in the classical HLA genes in the major histocompatibility complex (MHC, 6p21.3). Associations have been found both in class I [4] and class II [5–7] HLA genes, although the association with class II seems to be more essential. Associations vary from protective to predisposing alleles, or alleles influencing clinical outcomes. Our group has reported that in Finnish sarcoidosis patients class II *HLA-DRB1*03:01* and the haplotype *HLA-DRB1*04:01-DPB1*04:01* associate with a favorable disease outcome when compared to Finnish control population or between sarcoidosis prognosis groups [8,9]. The primary function of HLA-DR molecules is to present fragments of antigenic peptides to specific $CD4^+$ T cells. Peptide-binding groove is an important part of the antigen presentation. In sarcoidosis patients, vimentin peptide has been identified to be presented in HLA molecules [10]. It is hypothesized that vimentin could bind to HLA-DRB1*03 by using residues in pockets 1, 4, 6 and 9 [11]. HLA-DRB1*03 and HLA-DRB1*04 differ in peptide-binding amino acid sequences in these pockets (IMGT/HLA). Thus, association with *HLA-DRB1*03:01* and *HLA-DRB1*04:01-*

* Corresponding author. Transplantation Laboratory, Medicum, POBox 21 (Haartmaninkatu 3), FIN-00014, University of Helsinki, Finland.
E-mail address: Laura.lahtela@helsinki.fi (E. Lahtela).

https://doi.org/10.1016/j.rmed.2017.09.014
0954-6111/

*DPB1*04:01* might not be solely explained by antigen-presenting.

Angiotensin-converting enzyme (ACE) gene in chromosome 17q23 has also been associated with sarcoidosis in many studies. Most studied polymorphism in the *ACE* gene is the insertion, I and deletion, D variation [10,12]. Besides the I/D variation, little attention has so far been paid to the role of other *ACE* variants in relation to sarcoidosis [13]. Previously, our group wanted to extend the knowledge of the *ACE* gene in sarcoidosis by genotyping 29 single nucleotide polymorphisms (SNPs) spanning the gene from 5'upstream to 3'downstream [14]. These SNPs included tag SNP rs4343 for I/D polymorphism. We found a novel SNP (rs9905945) in the 5'upstream region of the *ACE* gene. Rs9905945 (C allele) associated with favourable disease prognosis in Finnish sarcoidosis patients further characterizing genetic differences between Finnish sarcoidosis patients with different clinical outcomes. However, when replicating in the Czech sarcoidosis patients, the SNP rs9905945 did not show an association with prognosis of sarcoidosis, possibly reflecting the population-specific genotype distribution.

The main objective of this study was to expand the previous results and analyse the combined influence of the *ACE* SNP rs9905945 with the protective HLA markers *HLA-DRB1*03:01* and *HLA-DRB1*04:01-DPB1*04:01* in Finnish sarcoidosis patients. The results might offer more accurate prognostic markers for evaluating the disease course in Finnish patients.

Study subjects and patient characteristics have been previously described in detail [9]. In summary, we examined a total of 188 Finnish patients recruited from 17 pulmonary units throughout the country with verified pulmonary sarcoidosis followed-up for 5–15 years and clinically categorized into subgroups based on disease prognosis. The patients were divided into those with a disease resolved within 2 years (n = 89) and to those with persisting activity after 2 years (n = 97). Disease activity after 2 years follow-up was determined using the generally accepted WASOG (World Association of Sarcoidosis and Other Granulomatous diseases) criteria. All the subjects gave their written informed consent to participate in the genetic association study.

The C allele of the *ACE* SNP rs9905945, *HLA-DRB1*03:01* and *HLA-DRB1*04:01-DPB1*04:01* were utilized for statistical analyses. Chi-square and Fisher's exact test when appropriate were used to assess significant differences in test marker frequencies (rs9905945, *HLA-DRB1*03:01* and *HLA-DRB1*04:01-DPB1*04:01*) between the groups (persistent disease, resolved disease). The effect of test markers for sarcoidosis prognosis was analyzed with logistic regression analysis (forward stepwise).

The C allele of the *ACE* SNP rs9905945 on the genotype level showed independent association with good disease prognosis when tested together with *HLA-DRB1*03:01* and the haplotype by logistic regression (Table 1.). When combining the frequencies of the rs9905945 C phenotypes and of the *HLA-DRB1*03:01* and the haplotype *HLA-DRB1*04:01-DPB1*04:01*, the strongest association was found for a combination of either/or both HLA markers and rs9905945 C allele for resolved disease prognosis (37.1% in resolved vs. 11.3% in persistent, p < 0.001, OR = 4.61, (95%CI 2.15–9.86)). When stratifying for HLA markers in persistent and resolved study groups, in the *HLA-DRB1*03:01* stratified group, the rs9905945 C phenotype did not show significantly different frequency between resolved and persistent groups (80.6% in resolved vs. 56.3% in persistent group, p = 0.096, OR = 3.24 (95%CI 0.86–12.3)). However, when comparing *HLA-DRB1*04:01-DPB1*04:01* stratified group, the frequency of rs9905945 C phenotype was more common in resolved group (87.5% in resolved vs. 22.2% in persistent group, p = 0.002, OR = 24.5 (95%CI 2.83–212.4)). Due to small sample sizes, the stratified groups were combined. In the combined group with either/or both HLA markers, the rs9905945 C phenotype remained significantly more common in resolved group (80.5% in resolved group vs. 44.0% in persistent group, p0.002, OR = 5.25 (95%CI = 1.74–15.8)).

In our previous studies, we have demonstrated that Finnish resolved and persistent sarcoidosis patients have different combinations of disease-related HLA markers (*HLA-DRB1*, *-DPB1* and *BTNL2* polymorphism). We showed that combinations of markers had higher distinction capability between severity of sarcoidosis than these markers alone, indicating the need for analyzing not just single association marker, but wider variety of predisposing or protecting factors. In our series, *HLA-DRB1*03:01* and the haplotype *HLA-DRB1*04:01-DPB1*04:01* positive patients are likely to develop sarcoidosis that will resolve spontaneously within two years. Our current study suggests that the novel association between rs9905945 and the good disease prognosis is independent of the *HLA-DRB1*03:01* allele and the *HLA-DRB1*04:01-DPB1*04:01* haplotype. However, the combination of the *ACE* SNP and HLA markers seems to enhance specificity to detect different disease outcomes compared to HLA markers alone.

To our knowledge, association between ACE and HLA molecules in sarcoidosis has not been found before. The aetiology of the disease is unknown and it is thought to involve a complex interplay between genes. Sarcoidosis is characterized by epithelioid granulomas in affected organs [15]. Current understanding is that antigen-presenting cells present peptides in a way that recognition by CD4 T lymphocytes initiates an inflammatory response resulting in granuloma formation [16] thus, explaining the HLA class II genes in sarcoidosis susceptibility. On the other hand, epithelioid cells in granulomas are the main source of ACE. High serum-ACE levels are

Table 1
Influence of significant good prognosis markers for Finnish sarcoidosis patients; *HLA-DRB1*03:01*, haplotype *HLA-DRB1*04:01-DPB1*04:01* and rs9905945 in the *ACE* gene region.

Influence of independent HLA markers on disease prognosis	Resolved[a]	Persistent[a]	Resolved vs. Persistent	
	%	%	p	OR (95% CI)
*HLA-DRB1*03:01* phenotype			0.006	2.74 (1.33–5.64)
*HLA-DRB1*04:01-DPB1*04:01* phenotype			0.043	2.96 (1.04–8.47)
rs9905945 C phenotype			0.041	2.07 (1.03–4.16)
Influence of marker combinations on disease prognosis				
*HLA-DRB1*03:01*	34.8	16.5	0.004	2.71 (1.36–5.40)
*HLA-DRB1*04:01-DPB1*04:01*	16.9	6.2	0.022	3.07 (1.14–8.32)
*HLA-DRB1*03:01/HLA-DRB1*04:01-DPB1*04:01*	44.9	22.7	0.001	2.78 (1.45–5.24)
rs9905945 C phenotype	79.8	66.0	0.035	2.03 (1.05–3.96)
*HLA-DRB1*03:01* + rs9905945 C phenotype	28.1	9.3	0.001	3.82 (1.67–8.73)
*HLA-DRB1*04:01-DPB1*04:01* + rs9905945 C phenotype	15.7	2.1	0.001	8.87 (1.95–40.23)
*HLA-DRB1*03:01/HLA-DRB1*04:01-DPB1*04:01* + rs9905945 C phenotype	37.1	11.3	<0.001	4.61 (2.15–9.86)

[a] Number of subjects: all, n = 186; resolved, n = 89; persistent, n = 97.

widely observed in sarcoidosis and are thought to correlate with granuloma mass and sarcoidosis activity [17] making the *ACE* gene a potential susceptibility factor as well. Epithelioid cells of mucosal surfaces have been shown to act as antigen-presenting cells, presenting MHC class II molecules [18]. Epithelioid cell of granulomas might therefore also present MHC II molecules, thus connecting these two pathways.

In conclusion, we discovered that a combination of the *ACE* SNP rs9905945 and previously reported HLA markers enhance the accuracy for predicting disease course in Finnish sarcoidosis patients further characterizing genetic differences between Finnish sarcoidosis patients with different prognosis. However, probable population specific distribution of the disease associated SNPs must be considered before its potential application to other populations.

Ethics statement

The study was performed with approval of Ethics Committee of the Department of Internal Medicine, Hospital District of Helsinki and Uusimaa, Finland.

Conflict of interest

The authors declare no known conflict of interest.

Funding

The study was supported by research grants from the Nummela Foundation of Clinical Research.

Acknowledgements

The authors thank Leena Saraste for assisting with language issues.

References

[1] P. Spagnolo, R.M. du Bois, Genetics of sarcoidosis, Clin. Dermatol 25 (3) (2007 May-Jun) 242–249.
[2] M.C. Iannuzzi, B.A. Rybicki, A.S. Teirstein, Sarcoidosis, N. Engl. J. Med. 357 (21) (2007 Nov 22) 2153–2165.
[3] J. Muller-Quernheim, M. Schurmann, S. Hofmann, K.I. Gaede, A. Fischer, A. Prasse, et al., Genetics of sarcoidosis, Clin. Chest Med. 29 (3) (2008 Sep), 391,414, viii.
[4] J. Grunewald, A. Eklund, O. Olerup, Human leukocyte antigen class I alleles and the disease course in sarcoidosis patients, Am. J. Respir. Crit. Care Med. 169 (6) (2004 Mar 15) 696–702.
[5] H. Sato, F.A. Woodhead, T. Ahmad, J.C. Grutters, P. Spagnolo, J.M. van den Bosch, et al., Sarcoidosis HLA class II genotyping distinguishes differences of clinical phenotype across ethnic groups, Hum. Mol. Genet. 19 (20) (2010 Oct 15) 4100–4111.
[6] J. Grunewald, B. Brynedal, P. Darlington, M. Nisell, K. Cederlund, J. Hillert, et al., Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients, Respir. Res. 11 (2010 Feb 26) 25.
[7] F. Mrazek, L.I. Holla, B. Hutyrova, V. Znojil, A. Vasku, V. Kolek, et al., Association of tumour necrosis factor-alpha, lymphotoxin-alpha and HLA-DRB1 gene polymorphisms with Lofgren's syndrome in Czech patients with sarcoidosis, Tissue Antigens 65 (2) (2005 Feb) 163–171.
[8] A. Wennerstrom, A. Pietinalho, J. Lasota, K. Salli, I. Surakka, M. Seppanen, et al., Major histocompatibility complex class II and BTNL2 associations in sarcoidosis, Eur. Respir. J. 42 (2) (2013 Aug) 550–553.
[9] A. Wennerstrom, A. Pietinalho, H. Vauhkonen, L. Lahtela, A. Palikhe, J. Hedman, et al., HLA-DRB1 allele frequencies and C4 copy number variation in Finnish sarcoidosis patients and associations with disease prognosis, Hum. Immunol. 73 (1) (2012 Jan) 93–100.
[10] J. Wahlstrom, J. Dengjel, B. Persson, H. Duyar, H.G. Rammensee, S. Stevanovic, et al., Identification of HLA-DR-bound peptides presented by human bronchoalveolar lavage cells in sarcoidosis, J. Clin. Invest. 117 (11) (2007 Nov) 3576–3582.
[11] J. Grunewald, Y. Kaiser, M. Ostadkarampour, N.V. Rivera, F. Vezzi, B. Lotstedt, et al., T-cell receptor-HLA-DRB1 associations suggest specific antigens in pulmonary sarcoidosis, Eur. Respir. J. 47 (3) (2016 Mar) 898–909.
[12] B. Rigat, C. Hubert, F. Alhenc-Gelas, F. Cambien, P. Corvol, F. Soubrier, An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels, J. Clin. Invest. 86 (4) (1990 Oct) 1343–1346.
[13] M. Schurmann, P. Reichel, B. Muller-Myhsok, T. Dieringer, K. Wurm, M. Schlaak, et al., Angiotensin-converting enzyme (ACE) gene polymorphisms and familial occurrence of sarcoidosis, J. Intern Med. 249 (1) (2001 Jan) 77–83.
[14] E. Lahtela, A. Wennerström, A. Pietinalho, M. Petrek, V. Kolek, M.-L. Lokki, O. Selroos, *ACE* gene variants and sarcoidosis in a Finnish population, Sarcoidoisis, Vasc. diffuse lung Dis. 34 (2017) 104–114.
[15] L.S. Newman, C.S. Rose, L.A. Maier, Sarcoidosis, N. Engl. J. Med. 336 (17) (1997 Apr 24) 1224–1234.
[16] P. Spagnolo, L. Richeldi, R.M. du Bois, Environmental triggers and susceptibility factors in idiopathic granulomatous diseases, Semin. Respir. Crit. Care Med. 29 (6) (2008 Dec) 610–619.
[17] M. Luisetti, A. Beretta, L. Casali, Genetic aspects in sarcoidosis, Eur. Respir. J. 16 (4) (2000 Oct) 768–780.
[18] D.J. Mulder, A. Pooni, N. Mak, D.J. Hurlbut, S. Basta, C.J. Justinich, Antigen presentation and MHC class II expression by human esophageal epithelial cells, Am. J. Pathol. 178 (2) (2011 Feb) 744–753 doi: 10.1016, http://www.ebi.ac.uk/ipd/imgt/hla.